Food Chemistry 185 (2015) 182–191

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Review

Coffee fermentation and flavor – An intricate and delicate relationship

Liang Wei Lee [a], Mun Wai Cheong [b], Philip Curran [b], Bin Yu [c], Shao Quan Liu [a,d,*]

[a] Food Science and Technology Programme, Department of Chemistry, National University of Singapore, S14 Level 5, Science Drive 2, Singapore 117542, Singapore
[b] Firmenich Asia Pte Ltd., 10 Tuas West Road, Singapore 638377, Singapore
[c] Agilent Technologies Singapore Pte Ltd., 1 Yishun Avenue 7, Singapore 768923, Singapore
[d] National University of Singapore (Suzhou) Research Institute, No. 377 Linquan Street, Suzhou Industrial Park, Suzhou, Jiangsu 215123, China

ARTICLE INFO

Article history:
Received 19 September 2014
Received in revised form 26 March 2015
Accepted 30 March 2015
Available online 2 April 2015

Keywords:
Coffee
Fermentation
Fungus
Flavor
Civet cat coffee
Extracellular enzymes

ABSTRACT

The relationship between coffee fermentation and coffee aroma is intricate and delicate at which the coffee aroma profile is easily impacted by the fermentation process during coffee processing. However, as the fermentation process in coffee processing is conducted mainly for mucilage removal, its impacts on coffee aroma profile are usually neglected. Therefore, this review serves to summarize the available literature on the impacts of fermentation in coffee processing on coffee aroma as well as other unconventional avenues where fermentation is employed for coffee aroma modulation. Studies have noted that proper control over the fermentation process imparts desirable attributes and prevents undesirable fermentation which generates off-flavors. Other unconventional avenues in which fermentation is employed for aroma modulation include digestive bioprocessing and the fermentation of coffee extracts and green coffee beans. The latter is an area that should be explored further with appropriate microorganisms given its potential for coffee aroma modulation.

Contents

1. Introduction

Coffee is ranked as the second most traded global commodity after petroleum in financial terms. In 2012, the total value of the coffee industry was estimated to be worth US $173.4 billion (International Coffee Organization (ICO), 2014a). In addition, global coffee consumption has also risen at an average annual rate of 1.9% over the past 50 years. This global appeal of coffee could be attributed to its desirable and alluring aroma.

The formation of coffee aroma occurs predominately during roasting via a complex series of Maillard reactions, caramelization and other thermal reactions involving aroma precursors that are

* Corresponding author at: Food Science and Technology Programme, Department of Chemistry, National University of Singapore, S14 Level 5, Science Drive 2, Singapore 117542, Singapore. Tel.: +65 516 2687; fax: +65 6775 7895.
E-mail address: chmLsq@nus.edu.sg (S.Q. Liu).

http://dx.doi.org/10.1016/j.foodchem.2015.03.124
0308-8146/

present in green coffee beans. Therefore, roasting is an avenue that has a significant impact on coffee aroma and unsurprisingly has been the main target for research into coffee aroma modulation over the past century. The focus of most studies within this area fall into two major categories: outlining of the roles in which aroma precursors in green coffee contribute towards coffee aroma formation (Poisson, Schmalzried, Davidek, Blank, & Kerler, 2009; Sunarharum, Williams, & Smyth, 2014; Yeretzian, Jordan, Badoud, & Lindinger, 2002) and the characterization of the impact of technical factors such as roasting temperature and time on the corresponding aroma profile (Baggenstoss, Poisson, Kaegi, Perren, & Escher, 2008; Petisca, Pérez-Palacios, Farah, Pinho, & Ferreira, 2013).

Besides roasting, brewing is another avenue at the consumer end that has a significant impact on coffee aroma. Consequently, there have been a reasonable amount of studies directed towards evaluating the impacts of brewing methods and parameters on coffee aroma (Gloess et al., 2013; Lopez-Galilea, Fournier, CID, & Guichard, 2006). Therefore, judging from the focus of the current available literature, research into the modulation of coffee aroma at the roasted coffee end is fairly saturated.

However, coffee aroma is affected by numerous factors from farm to cup (Sunarharum et al., 2014). Post-harvest coffee processing is another avenue which has a significant impact on coffee aroma but is still inadequately addressed (Bhumiratana, Adhikari, & Chambers, 2011). Analysis of the sensory profiles of dry- and wet-processed coffees found that the latter were more aromatic with fruity and acidic attributes and possessed lesser bitter, burnt and woody notes (Duarte, Pereira, & Farah, 2010; Leloup, Gancel, Liardon, Rytz, & Pithon, 2004). These differences in the sensory attributes could most likely be attributed to the fermentation process involved in mucilage removal in wet processing. However, the effects of fermentation during wet-processing on the aroma profile of coffee have not been fully elucidated and are often neglected since its main role is commonly accepted as mucilage removal.

The relationship between fermentation and the corresponding coffee aroma profile can be described as being intricate and delicate. With optimized parameters and appropriate starter cultures for fermentation during mucilage removal in wet-processing, fermentation can impart desirable attributes to the corresponding coffee aroma while uncontrolled fermentation inevitably leads to off-flavors (Gonzalez-Rios et al., 2007b; Jackels et al., 2006; Jackels & Jackels, 2005b; Lin, 2010). Furthermore, the extension of controlled fermentation to other unconventional avenues has also been found to bring about coffee aroma modulation. This would lead to greater flavor diversification which is evident in the beer and wine industry along with the emergence of fermented beverages of unconventional fruit origins (Marshall & Mejia, 2011). Therefore, the aim of this review is to illustrate the intricate and delicate relationship between fermentation and coffee aroma by summarizing current available studies that highlight the impacts of fermentation during coffee processing on the corresponding coffee aroma profile and other novel fermentation methodologies that bring about coffee aroma modulation.

2. Formation of coffee aroma

Aroma is a key attribute that defines the quality as well as the level of consumer acceptance for coffee products (De Maria, Trugo, Aquino Neto, Moreira, & Alviano, 1996). The formation of the desirable aroma of coffee is attributed to Maillard reactions, along with other thermally catalyzed reactions that occur during roasting at temperatures usually beyond 200 °C. Consequently, hundreds of thermally-derived volatile compounds have been identified in coffee aroma via various forms of head-space sampling methods such as static headspace extraction (Maeztu et al., 2001), solid-phase microextraction (SPME) (Cheong et al., 2013; Petisca et al., 2013), needle trap device (NTD) (Eom & Jung, 2013), headspace sorptive extraction (HSSE) and stir bar sorptive extraction (SBSE) (Bicchi, Iori, Rubiolo, & Sandra, 2002) that are usually coupled to gas chromatography/mass spectrometry (GC–MS) for analysis. The SPME method is nowadays commonly used as it provides a simple, solvent-less, robust and generally reproducible form of analysis of coffee aroma. Furthermore, SPME fibers of different sorbent types are commercially available. However, due to the poor partitioning between the sorbent and sample phase, it lacks sensitivity. On the other hand, HSSE and SBSE provide higher sensitivity compared to SPME but require a separate desorption unit to be attached to the GC instrument. Currently, multiple-SPME method which involves the extraction of one coffee sample with multiple SPME fibers followed by a one-time injection of all the volatiles extracted into the GC instrument is explored and developed so as to enhance the sensitivity of SPME (Lee, Lee, Lee, & Buglass, 2013). With the advancement of analytical technologies, analytical techniques such as two-dimensional gas chromatography coupled to either MS or flame ionization detector (FID) (Chin, Eyres, & Marriott, 2011) and nuclear magnetic resonance (NMR) spectroscopy (Ciampa, Renzi, Taglienti, Sequi, & Valentini, 2010) have been used for headspace and compositional analysis of roasted coffee aroma. However, only a fraction of the odorants detected from coffee aroma were determined to be character-impact odorants with flavor dilution (FD) values in the range of 16–2048. A list of these compounds is provided in Table 1 which consists of furanones, pyrazines, thiols, phenolic and sulfur compounds that are responsible for the caramelic, earthy, roasty, meaty, smoky, spicy, buttery, fruity attributes of roasted coffee.

2.1. Thermal degradation of aroma precursors

Sugars, proteins, amino acids and phenolic compounds are important aroma precursors present in green coffee beans which play an essential role in coffee aroma formation. During roasting, thermal degradation of polysaccharides and simple sugars are responsible for the formation of caramelization products. Similarly, chlorogenic acids together with other non-volatile phenolic derivatives such as 5-feruloylquinic acid are also readily hydrolyzed, yielding hydroxycinnamic acid derivatives. In addition, real-time mass spectrometry monitoring techniques also revealed that hydroxycinnamic acids such as ferulic acid undergo further decarboxylation and other chemical reactions, resulting in the formation of potent volatile phenolic compounds such as guaiacol, *p*-vinylguaiacol and phenols (Dorfner, Ferge, Kettrup, Zimmermann, & Yeretzian, 2003).

Trigonelline is an alkaloid present in green coffee beans that is also extensively degraded, yielding important coffee odorants such as pyridines and pyrroles. The impacts of pyrolysis of aroma precursors were highlighted in the study conducted by De Maria et al. (1996), where pyrolysis of hydroxy-amino acids such as threonine and serine occurring during roasting was another pathway responsible for the generation of alkylpyrazines derivatives and pyrroles. Significant pyridine formation was attributed to trigonelline degradation.

2.2. Maillard reactions

Maillard reactions is the main avenue for coffee aroma formation as it is responsible for the generation of various classes of coffee aroma-impact compounds such pyrazines, pyrroles, thiols, furanones, pyridines, and thiophenes. Therefore, given the primary role that it plays in the development of coffee aroma, much research emphasis has been placed on studying the mechanisms behind the formation of Maillard-derived aroma compounds. It

Table 1
A list of the potent odorants detected in roasted coffee and their respective concentrations.

Potent odorants in coffee (as identified in literature)	FD values[a]		Concentrations in coffee grounds in ppb	Sensory descriptors
	Coffee grounds	Coffee brew		
Acids				
2-Methyl-1-butanoic acid[a]	64	64	–	Sweaty, acidic[d]
3-Methyl-1-butanoic acid[a]	64	64	18,060–32,180[g]	Sweaty, acidic[d]
Furanonxes				
4-Hydroxy-2,5-dimethyl-3(2H)-furanone[a,b]	16	256	112,000–140,000[b,e]	Caramelic[a]
3-Hydroxy-4,5-dimethyl-2(5H)-furanone (Sotolon)[a,b,c]	512	2048	1360–1900[b,c,e]	Seasoning-like[a], spicy[f]
5-Ethyl-3-hydroxy-4-methyl-2(5H)-furanone[a,b]	512	1024	104[b]	Seasoning-like[a]
Ketones				
2,3-Butanedione[a,b]	16	32	48,400–49,000[b,e]	Buttery, caramel-like[d]
2,3-Pentanedione[a,b]	32	32	34,000–35,000[b,e]	Buttery, caramel-like[d]
2-Hydroxy-3,4-dimethyl-2-cyclo-penten-1-one[a]	64	128	–	Caramel-like[a]
Norisoprenoids				
(E)-β-damascenone[a,b,c]	2048	64	222–260[b,c,e]	Honey-like[a], fruity[d]
Phenolic compounds				
Guaiacol[a,b]	32	16	2400–3040[b,e]	Phenolic, burnt[a,d]
4-Ethylguaiacol[a,b,c]	256	512	1420–4060[b,c,e]	Spicy[a]
p-Vinylguaiacol[a,b,c]	512	512	39,000–55,200[b,c,e]	Spicy[a]
Vanillin[a,b,c]	32	512	3290–4100[b,c,e]	Vanilla-like[a]
Pyrazines				
3-Isopropyl-2-methoxypyrazine[a,c]	128	32	2.4[c]	Earthy, roasty[a]
2-Ethyl-3,5-dimethylpyrazine[a,b]	2048	1024	249–400[b,e]	Earthy, roasty[a,d]
2,3-Diethyl-5-methylpyrazine[a,b]	512	128	73–100[b,e]	Earthy, roasty[a]
3-Isobutyl-2-methoxypyrazine[a,b,c]	512	128	59–120[b,c,e]	Earthy[a]
Sulphur compounds				
Methional[a,b,c]	128	512	213–250[b,c,e]	Boiled potato-like[a]
Bis(2-methyl-3-furyl)disulphide[a]	32	128	–	Meat-like[f]
Thiols				
2-Furfurylthiol[a,b]	256	64	1680–1700[b,e]	Roasty, coffee-like[a]
3-Mercapto-3-methyl-l-butanol[a]	32	64	–	Meaty[a]
3-Mercapto-3-methylbutylformate[a,b]	2048	256	77–130[b,e]	Catty, roasty[a]

Potent odorants of roasted coffee and their respective FD values and concentrations were identified and collated from the following references.
[a] Blank, Sen, and Grosch (1992).
[b] Czerny, Mayer, and Grosch (1999).
[c] Czerny and Grosch (2000).
[d] Lopez-Galilea et al. (2006).
[e] Mayer, Czerny, and Grosch (2000).
[f] Mayer & Grosch (2001).
[g] Cheong et al. (2013).

was established in various studies elaborated as follows that the amino acids and reducing sugars generated from the extensive hydrolysis of proteins and sucrose during roasting are key flavor precursors that participate in Maillard reactions.

In a study conducted by Wong, Abdul Aziz, and Mohamed (2008), the Maillard reactions of a particular mixture of amino acids with glucose conducted in model systems yielded a sensory profile containing odor attributes that were characteristic of the amino acids present within the system. This could be explained by studies showing that the formation of specific potent odorants was very much correlated with the amino acid composition present within the system (Amrani-Hemaimi, Cerny, & Fay, 1995; Low, Parker, & Mottram, 2007; Poisson et al., 2009). A higher glycine and alanine content in model systems containing reducing sugars corresponded to an increase in alkylpyrazines formation.

Furthermore, the application of ^{13}C isotope labelling showed that amino acids, not only functioned as a nitrogen source, but also contributed carbon skeletons to the formation of alkyl-substituents on alkylpyrazines. Given that the formation of alkylpyrazines from different amino acids occurs via different mechanisms, the amino acid profile of green beans would have a significant impact on the alkylpyrazines formation. The involvement of amino acids as flavor precursors in Maillard reactions for coffee aroma formation was reiterated by another study by Montavon, Mauron, and Duruz (2003). Changes to the green coffee protein profile were observed using two-dimensional polyacrylamide gel electrophoresis and were attributed to the complete and consistent fragmentations of protein during roasting to form amino acid precursors.

Reducing sugars also play a significant role in Maillard reactions. In the study conducted by De Maria et al. (1996), ethanol-soluble and -insoluble fractions of green coffee beans were isolated and the constituents present within the individual fractions were evaluated for the roles they played in coffee aroma formation. In the ethanol-soluble fraction, complete sucrose degradation corresponding to significant formation of furans following roasting highlighted the role of sucrose hydrolytic products in Maillard reactions. The absence of sucrose in the ethanol-insoluble fraction expectedly yielded significantly lower concentrations of furans, possibly generated from other pyrolytic pathways. Reducing sugars also play a significant role in furanone generation, as the formation of 4-hydroxy-2,5-dimethyl-3[2H]-furanone was not observed in systems where sugars were absent (Poisson et al., 2009). Furthermore, different reducing sugars were found to exhibit different reactivity rates in Maillard reactions, with fructose being shown to be a more efficient precursor compared to glucose. Isotope-labeling of sucrose ($^{13}C_6$-fructose–$^{12}C_6$-glucose) showed that there was complete and efficient conversion of fructose into 4-hydroxy-2,5-dimethyl-3[2H]-furanone when subjected to coffee roasting conditions as opposed to glucose (Poisson et al., 2009).

Therefore, the above studies extensively highlighted the importance of the roles in which proteins, carbohydrates and their respective hydrolytic products play as key flavor precursors in the formation of potent odorants of coffee aroma from Maillard reactions.

2.3. Relationship between the non-volatile profiles of green coffee and the aroma profiles of roasted coffee

Consequently, it can be deduced that the volatile profiles and subsequently, the aroma profiles and cupping qualities of roasted coffee are very much dependent on the composition of aroma precursors present in green coffee beans prior to roasting. Such a notion was substantiated by studies which showed that differences in the concentrations of aroma precursors such as proteins, carbohydrates and chlorogenic acids in green coffee beans of the same cultivar corresponded to different cupping qualities after roasting (Franca, Mendonça, & Oliveira, 2005; Franca, Oliveira, Mendonça, & Silva, 2005). In a study by Farah, Monteiro, Calado, Franca, and Trugo (2006), the cupping quality was found to be positively correlated with the levels of aroma precursors like trigonelline and 3,4-dicaffeoylquinic acid but negatively correlated with the concentrations of mono-esters of phenolic constituents like 3-caffeoylquinic acid present in green coffee beans prior to roasting.

Therefore, since aroma precursors in green coffee beans play an essential role in the formation of volatile compounds associated with coffee aroma during roasting (Sections 2.1 and 2.2), differences in the concentrations of aroma precursors will indirectly correspond to differences in the volatile and aroma profiles of roasted coffee. This inter-dependent relationship between the non-volatile constituents of green coffee beans and the corresponding aroma profile of roasted coffee fuels the notion that changes to the non-volatile composition in green coffee beans brought about by processes along the processing chain such as fermentation could have an eventual impact on coffee flavor.

3. Coffee processing

The post-harvest treatment of coffee cherries is a notable avenue which confers significant but variable impacts on volatile and aroma profiles of roasted coffee. Coffee is prepared from the pair of seeds located centrally within the coffee cherry after the exocarp, mesocarp and the mucilage layer which is a colorless and viscous pectin layer located beneath the mesocarp are removed during processing. Depending on the area of production and species, coffee cherries are processed via one of the two methods mentioned by Schwan and Wheals (2003). In regions like Colombia, Central America and Hawaii, Arabica coffees are processed via the wet method where red mature coffee cherries of consistent maturity are hand-picked and mechanically depulped to remove the exocarp and mesocarp. Subsequently, the thin mucilaginous layer surrounding the coffee seeds are removed via a fermentation process of approximately 24–48 h. Finally, the processed coffee beans are then mechanically- or sun-dried to a moisture content of 11–12% so as to achieve microbial stability.

On the other hand, the dry method, also known as natural processing, is commonly employed for Robusta coffees, as well as in countries like Brazil and Ethiopia which expect extended periods of sunshine. For the dry method, coffee cherries are either hand-picked or machine-harvested when most of them are matured. Consequently, the levels of maturity are not consistent among the harvested coffee cherries. Following harvesting, coffee cherries are then left to dry under the sun in layers of approximately 10 cm for 10–25 days, where they are constantly heaped and re-spread. During the process, natural microbial fermentation and enzymatic actions lead to the breakdown of the pulp and mucilage with the coffee cherry intact. At the end of the drying process, the dried exocarp is removed, yielding coffee beans of 10–11% moisture content (Schwan & Wheals, 2003).

Based on the statistics obtained from major coffee-exporting countries, only 9% of the coffees produced in the calendar year of 2013/2014 were processed via the wet method (International Coffee Organization, 2014b). Nevertheless, with proper control over the process parameters, the wet method generates fewer defective beans and is capable of preserving the intrinsic qualities of the coffee beans, thereby producing coffees of superior aroma qualities compared to the dry method.

3.1. Impacts of processing methods on coffee aroma

The impacts of processing methods on coffee aroma are evident as quality differences have been observed in identical coffee samples processed via the two processing methods in parallel (Selmar, Bytof, & Knopp, 2002). The chemical analysis of differentially processed green coffee beans showed that free low molecular weight sugars such as fructose, glucose, arabinose and galactose were present at significantly lower levels in wet-processed coffees compared to dry-processed coffees while the former contained significantly higher levels of glutamic and aspartic acids (Bytof, Knopp, Schieberle, Teutsch, & Selmar, 2005; Knopp, Bytof, & Selmar, 2005). The impacts of post-harvest treatments on the concentrations of aroma precursors such as 3-CQA, sucrose, free amino acids present in different Arabica coffee cultivars are presented in Table 2. It was observed that different types of coffee processing methods led to significant differences in the concentrations of free amino acids, reducing sugars and phenolic compounds present in green coffee beans of the same varietal (Arruda et al., 2012). Furthermore, analysis of the polysaccharide fractions isolated from differentially processed green coffee beans revealed differences in the monosaccharide contents (rhamnose, arabinose, galactose and mannose). This was attributed to the different extents of influence to which different processing methods have on the structural aspects of polysaccharides and the degradation of galactomannans that were present in green coffee beans (Tarzia, dos Santos Scholz, & de Oliveira Petkowicz, 2010). These are important coffee aroma precursors and differences in the concentrations of these compounds would therefore be responsible for the aroma quality differences that were observed. While differences in the chemical compositions of wet- and dry-processed green coffee beans have been documented in detail, the extent to which these changes to the non-volatile profiles translated into aroma profile differences between differentially-processed coffees and the processes that were responsible for these changes are less understood.

The changes in the chemical composition of green coffee beans would likely be brought about by metabolic activities that occurred during the course of coffee processing. Based on expression studies of germination-specific isocitrate lyase (ICL) and the analysis of β-tubulin, a key marker of cell division, it was established that germination was one of the metabolic processes that were responsible for these compositional differences (Selmar, Bytof, Knopp, & Breitenstein, 2006). This was supported by evidence showing the increase in the concentrations of free amino acids and γ-aminobutyric acid in processed green coffee since storage proteins were hydrolyzed to generate raw materials for the germination process (Bytof et al., 2005). The lower concentrations of galactomannans in processed green coffee beans were attributed to hydrolysis brought about by endo-β-mannanases, β-mannosidases and galactosidase (Tarzia et al., 2010). Furthermore, the decrease in the concentrations of simple sugars such as glucose and fructose (Joët et al., 2010; Knopp et al., 2005) could be attributed to sugar metabolism and inter-conversion expected during seed-germination process.

Table 2
Effects of post-harvest treatments on the composition of aroma precursors in different Arabica coffee cultivars.

Aroma precursors	Arabica cultivars	Concentration/g 100^{-1} g dry wt. of green coffee		
		Dry processing	Semi-dry processing	Wet processing
Sugars				
Sucrose	Yellow Bourbon[A]	–	10.05 ± 0.01	10.88 ± 0.04
	Red Catuaí[A]	–	11.68 ± 0.01	9.85 ± 0.03
	Rubi[A]	–	11.98 ± 0.02	9.16 ± 0.05
	Topázio[A]	–	12.66 ± 0.02	8.49 ± 0.04
Reducing sugars	Yellow Catuaí[B]	0.94 ± 0.03[a]	0.52 ± 0.02[b]	0.14 ± 0.01[c]
Total sugars	Yellow Catuaí[B]	7.98 ± 0.24[a]	7.54 ± 0.19[a]	8.98 ± 0.32[b]
Nitrogenous compounds				
Caffeine	Yellow Bourbon[A]	–	1.13 ± 0.02	1.05 ± 0.02
	Red Catuaí[A]	–	1.13 ± 0.02	1.26 ± 0.02
	Rubi[A]	–	1.21 ± 0.01	1.19 ± 0.02
	Topázio[A]	–	1.43 ± 0.01	1.16 ± 0.01
Trigonelline	Yellow Bourbon[A]	–	0.09 ± 0.01	0.89 ± 0.01
	Red Catuaí[A]	–	0.78 ± 0.04	0.92 ± 0.01
	Rubi[A]	–	0.74 ± 0.01	0.80 ± 0.01
	Topázio[A]	–	0.92 ± 0.01	0.85 ± 0.02
	Yellow Catuaí[B]	1.17 ± 0.04[a]	1.13 ± 0.05[a]	1.37 ± 0.08[b]
Total free amino acids	Yellow Catuaí[B]	1.15 ± 0.07[a]	0.76 ± 0.02[b]	0.67 ± 0.02[c]
Total protein	Yellow Catuaí[B]	10.60 ± 0.36[a]	11.70 ± 0.50[b]	12.50 ± 0.50[b]
Phenolic compounds				
3-CQA	Yellow Bourbon[A]	–	0.40 ± 0.04	0.50 ± 0.02
	Red Catuaí[A]	–	0.44 ± 0.04	0.53 ± 0.02
	Rubi[A]	–	0.45 ± 0.02	0.42 ± 0.02
	Topázio[A]	–	0.54 ± 0.02	0.38 ± 0.01
	Yellow Catuaí[B]	0.42 ± 0.03[a]	0.41 ± 0.01[a]	0.49 ± 0.03[b]
Total phenolic content	Yellow Catuaí[B]	3.49 ± 0.05[a]	3.64 ± 0.06[b]	3.81 ± 0.08[c]

The concentrations of the respective aroma precursors present in different Arabica coffee cultivars subjected to different forms of post-harvest treatments were collated from the following literature [A](Duarte et al., 2010); [B](Arruda et al., 2012). Values with different lower case letters (a–c) in the same row indicate significant statistical differences. "–" indicates that data was not available from the literature. 3-CQA denotes 3-caffeoylquinic acid.

However, the extent of the metabolic activities that take place during the germination process was very much dependent on the coffee processing method and was determined using germination markers like ICL and β-tubulin (Selmar et al., 2006). The expression of ICL and β-tubulin peaked during the fermentation process in wet-processing and decreased during the drying stage. On the other hand, for dry-processing, the expression of ICL and β-tubulin remained low during the initial stages of processing and increased only 6 days following the onset of processing. Consequently, it was further established that the aroma quality differences between wet- and dry-processed coffees were attributed to the metabolic processes that were specific to each type of post-harvest treatment (Selmar et al., 2006). The superior aroma qualities of wet-processed coffees could thus be due to the physiological processes promoted by the high ICL expression during the fermentation process and this highlighted the importance of fermentation.

Another study by Gonzalez-Rios et al. (2007a) showed that the fermentation process in wet-processing was responsible for the comparatively superior aroma qualities of wet-processed coffees. The impacts of different combinations of pulping and mucilage removal methods on coffee aroma profiles were studied. The post-harvest treatment consisting of pulping with a disc pulper followed by fermentation in water to facilitate the removal of mucilage removal produced coffees with desirable traits such as fruity, flora and caramelic attributes. However, when dry fermentation was used for mucilage removal instead, coffees were characterized with buttery and nutty attributes and did not possess the above-mentioned desirable traits. When a mechanical mucilage remover was used semi dry-processing), coffees produced were then characterized with unpleasant attributes like sour, toasted and bitter almond notes. Coffees that were produced from treatments where only the method of pulping was varied gave relatively similar olfactory profile. Such a study showed that the fermentation process during processing has significant impacts on the corresponding coffee aroma.

It was also illustrated that the superior aroma characteristics of wet-processed coffees could be attributed to the production of microbial metabolites which were aroma precursors of roasted coffee during fermentation (Mussatto, Machado, Martins, & Teixeira, 2011).

4. Fermentation in coffee processing

However, the impacts of fermentation on coffee aroma profiles are often neglected as its main role in coffee processing is to facilitate the removal of the mucilage layer. The mucilaginous layer of the depulped coffee beans comprises of 84.2% water, 8.9% protein, 4.1% sugar, 0.91% pectic substances and 0.7% ash (Belitz, Grosch, & Schieberle, 2009). Further analysis of its polysaccharide composition revealed that the alcohol-insoluble component constituted 30% pectins, 8% cellulose, and 18% neutral non-cellulosic polysaccharides which consist of monosaccharides such as arabinose, xylose and galactose and other simple sugars (Avallone, Guiraud, Guyot, Olguin, & Brillouet, 2006) regardless of the way in which the mucilage layer was removed. Pectins consisted of predominantly galacturonic acids with high extent of methylation and moderate levels of acetylation. After the coffee cherries are mechanically depulped, these compounds are degraded and metabolized to a certain extent during the fermentation process in wet-processing. It was hypothesized that such metabolism during fermentation could result in the generation of an osmotic gradient originating from the exterior into the interior of the mucilage layer which disrupted its attachment to the coffee parchment (Avallone et al., 1999). Subsequently, the remaining coffee parchment is dried and hulled.

4.1. Microbiology of coffee fermentation

Since the mid-1900s, numerous species of microorganisms have been isolated from the fermentation phase of wet-processing (Agate & Bhat, 1966; Avallone, Guyot, Brillouet, Olguin, & Guiraud, 2001; de Melo Pereira et al., 2014; Masoud, Cesar, Jespersen, & Jakobsen, 2004; Masoud & Kaltoft, 2006; Pederson & Breed, 1946; Van Pee & Castelein, 1972). Aerobic bacteria such as *Klebsiella ozaenae*, *K. oxytoca*, *Erwinia herbicola*, *E. dissolvens*, *Hafnia* spp., *Enterobacter aerogenes* and lactic acid bacteria such as *Leuconostoc mesenteroides*, *Lactobacillus brevis* are the bacterial species that were isolated from the fermentation process. Yeast species such as *Kloeckera apis apicualata*, *Candida guilliermondii*, *C. tropicalis*, *C. parapsilosis*, *Cryptococcus albidus*, *C. laurentii*, *Pichia kluyveri*, *P. anomala*, *Hanseniaspora uvarum*, *Saccharomyces cerevisiae*, *Debaryomyces hansenii*, *Torulaspora delbrueckii* and *Rhodotorula mucilaginosa* have also been identified.

Furthermore, the isolation of pectinolytic microflora from the coffee fermentation process would suggest that their role in mucilage degradation. However, it is still not well understood if pectinolytic bacteria or yeasts are responsible for the degradation process. In most of the studies, the pectinolytic microflora that were identified include bacterial species such as *Klebsiella* spp., *Erwinia* spp., *Aerobacter* spp., *Escherichia* spp., *Bacillus* spp. and yeast species such as *S. marxianus*, *S. banyanus*, *S. cerevisiae* and *Schizosaccharomyces* spp. The absence of pectinolytic yeasts in one study conducted by Avallone et al. (2001) could be due to the use of a selective pectin media for identification. Some of the pectinolytic yeast species involved in coffee fermentation like *Kluyveromyces marxianus* and *S. cerevisiae* are not capable of utilizing pectin as the sole carbon source. As the fermentation phase proceeded, the yeast and bacterial population grew while the pectinolytic microflora population remained constant. However, as pH decreased in the later stages of fermentation, the yeast population would be the dominant microflora given its high acid tolerance (Avallone et al., 2001).

4.2. Issues with coffee fermentation during wet-processing

The main issue revolving around fermentation in wet-processing, which most studies agree upon, is the lack of controllability of the process. Even though the role in which fermentation in wet-processing play in improving coffee aroma quality may be contentious, there is no doubt that a poor control of the fermentation process would have a negative impact on coffee aroma. Over-fermentation is one of the most cited problems among coffee producers (Jackels & Jackels, 2005a, 2005b; Lopez, Bautista, Moreno, & Dentan, 1989; Puerta-Quintero, 2001).

Commonly, the end-point of the fermentation process is judged based on observations which are very subjective. Jackels and Jackels (2005a) pointed out that an object was used to generate a gap within the fermentation mass and the fermentation process was deemed to be completed if the gap was maintained due to friction between the coffee beans. In the event where the fermentation end-point is misjudged, under- or over-fermentation of the coffee parchment arises. Under-fermentation refers to the state at which there is no complete degradation of the mucilage layer which could subsequently promote the growth of undesirable microorganisms.

On the other hand, over-fermentation results in the production of black or "stinker" beans with poor visual and aroma characteristics. These beans are commonly associated with fruity, flora, sour and alcoholic attributes (Jackels & Jackels, 2005a). Therefore, this shows that there is only a fine margin between the fermentation process and the quality of coffee aroma. Consequently, time and temperature were pointed out in the Food and Agriculture Organization (2006) report to be crucial parameters of the fermentation process in wet-processing.

In addition, the reliance on the indigenous microflora that are present within the coffee cherries for fermentation during wet-processing contributes to its lack of controllability. Studies have shown that the microflora population present within the coffee parchment is dense and diverse, with numerous populations of yeasts, bacteria and filamentous fungi. More importantly, the microflora population was found to be dependent on the processing method involved and the processes involved in processing (Silva, Batista, & Schwan, 2008; Silva, Schwan, Sousa Dias, & Wheals, 2000). Consequently, this contributes to the inconsistency in the progress of the fermentation process and the aroma qualities of the processed coffees.

4.3. Optimization of coffee fermentation

In view of the issues surrounding coffee fermentation and its significant impacts on coffee aroma quality, there have been optimization studies conducted to gain controllability and consistency over the process from two main areas. These areas include the development of a methodology for the accurate determination of the end-point of fermentation and the use of a relevant starter culture for the fermentation process.

In a field study conducted in Nicaragua, it was established that chemical measurement of pH was a potentially reliable parameter to gauge the progress and end-point of fermentation (Jackels & Jackels, 2005b). The progress and end-point of fermentation was characterized by a decrease in pH of the fermentation mass from around 5.5–4 and this was consistent with numerous replicates of different batch sizes as well as in other studies (Rothfos, 1985; Velmourougane, 2012). Subsequently, in a separate study, the impacts of the end-point pH on the quality of coffee aroma were further evaluated by halting fermentation batches at different pHs of 4.6, 4.3 and 3.9 (Jackels et al., 2006). Based on the cupping results of the respective batches of roasted coffee beans, it was concluded that a lower end-point pH corresponded to a decrease in cupping quality and this was attributed to over-fermentation.

Therefore, based on these studies, pH measurements are a possible reliable tool for coffee producers to gain greater consistency and controllability over the coffee fermentation process, thus preventing under- or over-fermentation which has negative impacts on the quality of coffee aroma. It was shown that, when there is proper control of fermentation parameters such as the temperature range and humidity levels of the fermentation environment and accurate determination of the end-point pH, the fermentation process in wet-processing can impart desirable coffee aroma qualities (Lin, 2010; Velmourougane, 2012).

Besides pH measurements, starter cultures as well as pectinolytic enzyme treatment could be employed to gain greater consistency in the fermentation process. Studies have been conducted to investigate the impacts of incorporating enzymatic treatments and selected microbial starter cultures in coffee demucilization on the subsequent coffee aroma quality.

The co-inoculation of cellulase and *Aspergillius niger* significantly reduced the time required for mucilage degradation (30 min) compared to the other combinations which consisted of cellulase with microbial species such as *Rhizopus oryzae*, *Ln. mensenteroides* and *K. lactis* (24 h) (Lin, 2010). Furthermore, a higher concentration of reducing sugars was obtained following fermentation, presumably from the degradation of the mucilage layer, and this had a positive impact on coffee aroma since sugars are important aroma precursors for caramelization and Maillard reactions during roasting. Green coffee beans with higher reducing sugar content following fermentation were found to exhibit greater extent of caramelic, rich and sweet attributes.

In another study, the employment of pectinolytic enzyme treatment for coffee demucilization was found to generate slight sweet and acidic attributes coupled with a good body as opposed to the slight bitter, medicinal and woody attributes detected with natural fermentation, and slight bitter and harsh attributes obtained when mucilage layer was removed via mechanical means (Velmourougane, 2011).

Recently, there are two studies in which indigenous and non-indigenous bacterial and yeast species showing pectinolytic activity were screened for their suitability to act as starter cultures for the fermentation process in coffee processing (de Melo Pereira et al., 2014; Silva et al., 2013). The microorganisms were screened based on the polygalacturonase, pectin lyase and pectin methylesterase activities they exhibited.

Generally, potential starter cultures that were identified include yeast species such as *Saccharomyces* spp., *Pichia* spp. and *Candida* spp. which showed higher pectinolytic enzyme activity for efficient mucilage degradation during fermentation. Subsequently, the selected starter cultures were evaluated for their ability to enhance

the quality of coffee fermentation in wet, dry and semi-dry processing and produced coffees with distinctive flavor (de Melo Pereira et al., 2014; Evangelista, da Cruz Pedrozo Miguel, et al., 2014; Evangelista, Silva, et al., 2014). It was found that the employment of a selected culture for fermentation during coffee processing enhanced the quality of coffee aroma compared to coffees produced from fermentation involving indigenous microflora. The former possessed volatile compounds such as acetaldehyde, ethanol, ethyl acetate, isoamyl acetate and desirable traits such as caramel, fruity, buttery attributes that were characteristic of individual yeast starter cultures with the inherent characteristics of the coffees remaining unaffected. These results show that the employment of controlled fermentation through the use of pure starter cultures for the fermentation process in coffee processing promote consistency and controllability over the fermentation process.

5. Novel means of coffee aroma modulation via fermentation

Several different novel methods of coffee aroma modulation involving fermentation have been reported in the literature and are summarized in Fig. 1. Digestive bioprocessing is another method of processing that is gaining prevalence and is responsible for the production of Kopi Luwak, one of the most expensive coffees in the world. Kopi Luwak originates from the Indonesian islands of Java, Sumatra and Sulawesi and commands a hefty price of US $500 per pound as a result of its unique method of production and limited supply (Marcone, 2004). It is brewed from coffee beans that have been subjected to a combination of acidic, enzymatic and fermentation treatment as they transverse through the gastrointestinal tract of the civet cat.

Analysis by Marcone (2004) found evidence of protein hydrolysis which was attributed to the permeation of digestive enzymes and gastric juices through the endocarp of coffee cherries and bean surface as they transverse through the gastrointestinal tract of the animal. Changes to the amino acids composition would in turn have a significant impact on coffee aroma as amino acids are important aroma precursors in roasting. Protein hydrolysis would also account for the decrease in bitterness in the final brew while cupping results also revealed that Kopi Luwak was associated with lower body and acidity. Marcone (2004) also floated the idea that the characteristic flavor of Kopi Luwak could be attributed to a unique form of wet-processing given the similarity in acidification and fermentation processes taking place in digestive bioprocessing in civet cat and traditional wet-processing.

Another product of digestive bioprocessing is black ivory coffee which was introduced recently. It is the most expensive coffee in the world (US $800 per pound) and is brewed from coffee beans that are gathered from elephant dung after passing through its gastrointestinal tract. Generally, the processes in which the coffee beans are subjected to are somewhat similar to civet-cat coffee. However, the extent of the acidic, enzymatic and fermentation treatment may differ from civet-cat coffee beans given the difference in structural properties of the gastrointestinal tract and the microflora that are involved (Main, 2014). Currently, there are no studies on black ivory coffee. Nevertheless, it is an area that should be further explored so as to elucidate the impacts of the biochemical and structural properties of the animal's gastrointestinal tract on the corresponding coffee aroma profile.

Monsooning is another form of processing in which fermentation is responsible for the development of characteristic and desirable flavor attributes in monsooned coffee (Ahmad, Tharappan, & Bongirwar, 2003). It is mainly practiced in India, where fermentation occurs on the surface of beans as they are transported during the monsoon period. In one study, the microflora and enzyme

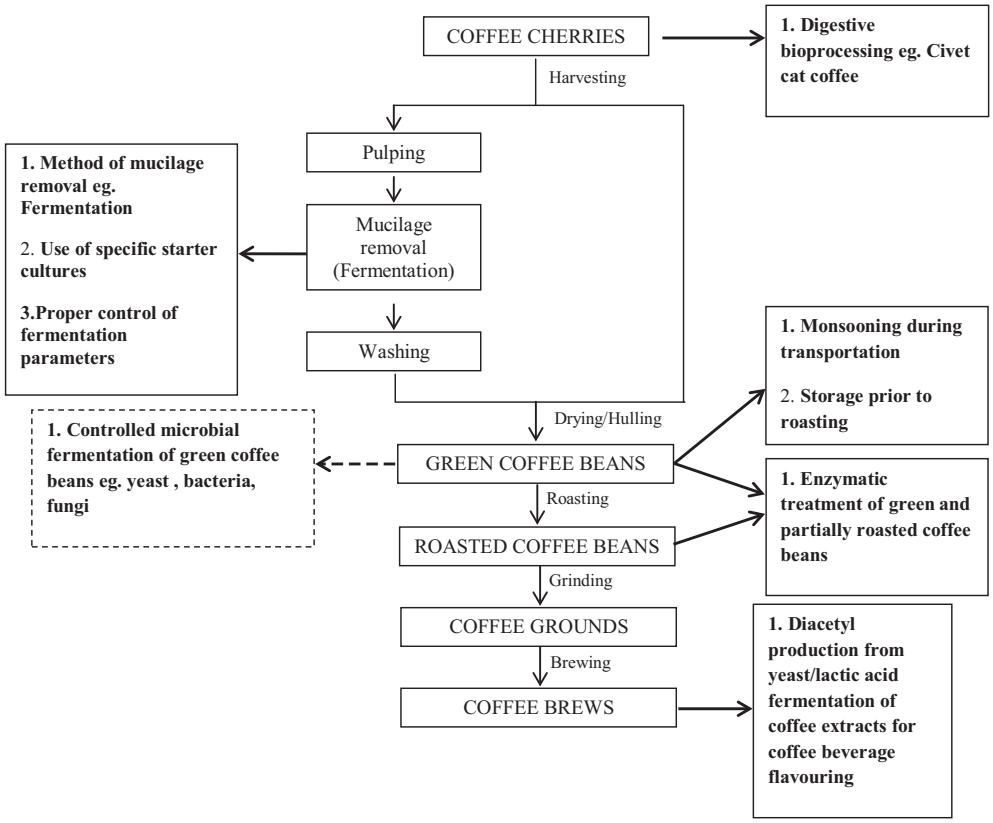

Fig. 1. Avenues from post-harvest to cup in which fermentation leads to or could potentially bring about coffee aroma modulation. Processes that have been reported in the literature are represented in solid lines while dashed lines represent potential avenues that should be further explored.

populations of monsooned and non-monsooned coffees were compared (Ahmad & Magan, 2002). It was observed that the bacterial (10^8 colony-forming units (CFU)/g) and fungal populations (10^5 CFU/g) were largest in monsooned coffees. The predominant fungal species were identified to be *Aspergillus* spp., *Penicillium* spp., *Absidia* spp., *Syncephalastrum* spp., *Mucor* spp. and *Rhizopus* spp. However, the roles in which these microorganisms play in aroma development remain to be elucidated.

There have also been patents utilizing fermentation for coffee aroma modulation. Boniello et al. (1989) described a process in which diacetyl, produced from lactic acid bacteria or yeast fermentation of a nutrient media containing water and soluble coffee substrates, was recovered and incorporated into coffee beverages or other coffee products to enhance their dairy or winey attributes. In the other patents that were filed, coffee extracts have been used as fermentation substrates for aroma modulation.

Yeast and bacterial fermentation of sugar-containing coffee extracts were employed for the flavor development in ready-to-serve coffee beverages (Baensch, Rippstein, & Wood, 1996; Shigeru, Toru, & Keiji, 1992). However, such a fermentation process would generate a small amount of alcohols which is considered undesirable for such beverages. Even though alcohol production could be limited with aeration, it would also lead to the loss of aroma compounds.

In another patent filed by Duboc and Milo (2003), alcohol production was inhibited by conducting fermentation at temperatures below 22 °C and selecting appropriate yeast strains that do not produce alcohols under these optimized conditions for the fermentation of sugar-containing coffee extracts. Fruity and floral attributes were obtained from the yeast/lactic acid bacterial fermentation of coffee extracts and they were attributed to the conversion of 2- and 3-methylbutanal to their corresponding alcohols and thiols compounds to thioacetates and diketones during fermentation (Duboc & Milo, 2003).

Consequently, fermentation has been shown to be capable of producing coffees with desirable traits. However, the above studies on coffee aroma modulation mainly alter the composition of preformed coffee aroma extract to induce desirable traits. There are studies which target coffee cherries but these substrates are more complicated to work with, given their high perishability. Thus, it would be interesting to apply the controlled fermentation process to commercial green coffee beans for coffee aroma modulation (represented in Fig. 1 with dashed lines). This is a novel method of processing in which the composition of aroma precursors in

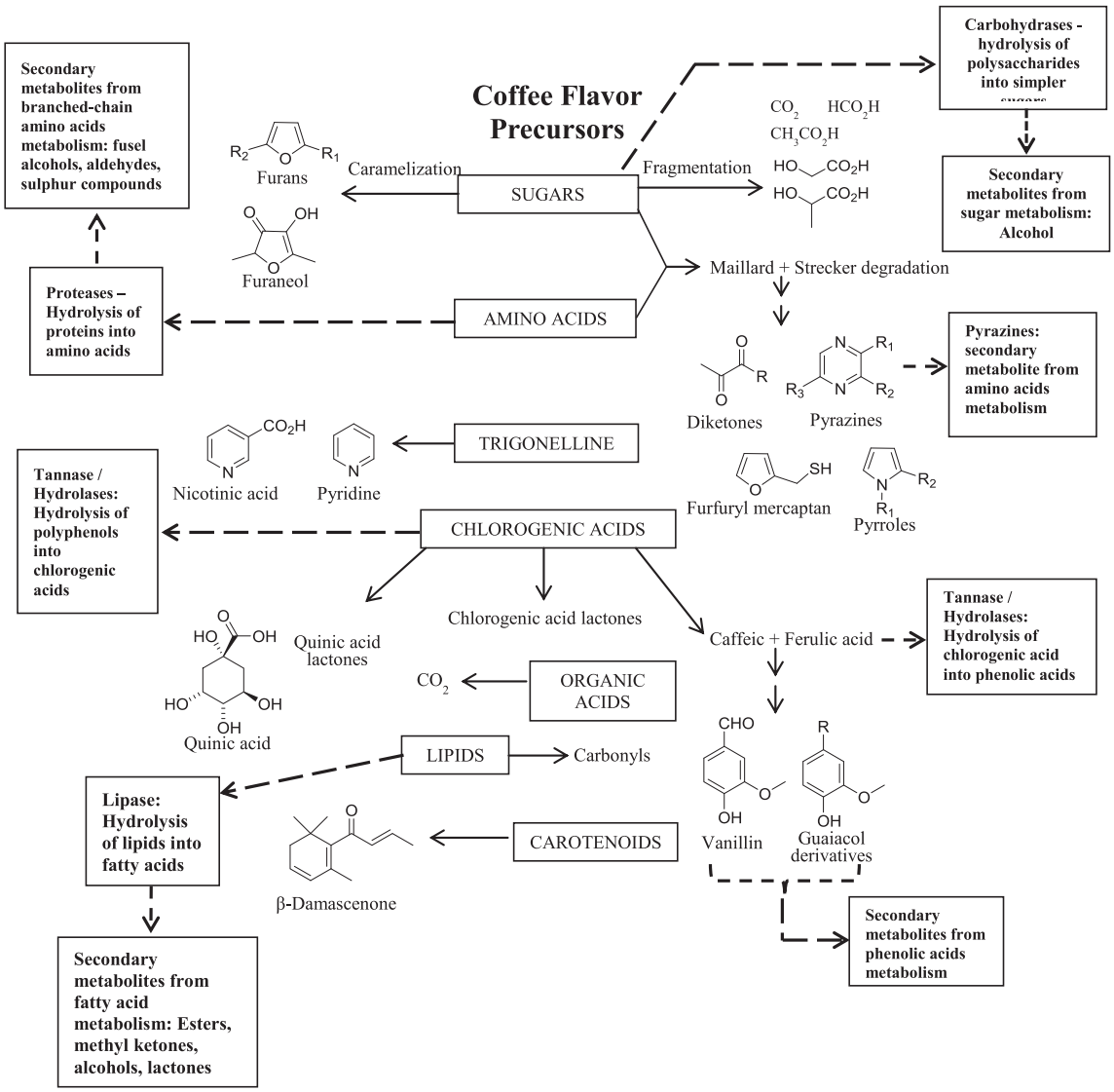

Fig. 2. An overview of the roles of precursors in coffee aroma formation during roasting with the possible modification of precursors composition and generation of secondary metabolites by controlled microbial fermentation represented in dashed lines (modified after Yeretzian et al., 2002).

green coffee beans is targeted and modified to bring about modulation of the corresponding coffee aroma profile as a result of changes in the extent of complex interactions of aroma precursors during roasting. A summary of the possible and expected changes to the composition of aroma precursors brought about by microbial fermentation is presented in dashed lines in Fig. 2. The extracellular enzymes and organic acids produced from fungi/yeast and lactic acid fermentation respectively could potentially lead to the hydrolysis of macromolecules such as carbohydrates, proteins and polyphenols, generating important aroma precursors such as reducing sugars, amino acids and chlorogenic acids. Secondary metabolites, produced during the course of fermentation, could also directly or indirectly coffee aroma.

Currently, there are only two patents utilizing such a concept for coffee aroma modulation. In the patent filed by Small and Asquith (1989), green and partially-roasted coffee beans were subjected to treatment by polysaccharide-degrading enzymes, proteases, lipases and phenol oxidase at pressure conditions above 1.7 MPa. The treated coffee beans were then dried and roasted. It was reported that coffees processed via such a method possessed desirable flavor attributes with a lower extent of bitterness.

On the other hand, in the patent filed by Li, Li, and Li (2010), coffee aroma modulation was achieved via solid-state fermentation of green coffee beans by *Antrodia camphorate*, a macrofungus under aseptic conditions for 15–60 days at 15–30 °C. It was reported that aroma compounds such as terpenes and sesquiterpenes were generated through such a process and this altered the aroma attributes of roasted coffee. Furthermore, under these conditions, the production of undesirable metabolites and flavor attributes could be readily avoided as compared to the civet-cat treatment. For civet-cat treated coffee beans, they were subjected to natural fermentation by microflora that are present within the gastrointestinal tract of the palm civet cat, which could easily lead to unwanted fermentation given the lack of controllability.

In addition, such a processing method would appeal more to the consumers. However, no analytical data and discussions were presented for both patents and there were no other research studies working in this area. Nevertheless, it would be interesting to investigate the impacts of solid-state fermentation of green coffee beans with microorganisms commonly used for aroma modulation in other food products like *Geotrichum candidum*, *R. oligosporus* and *Lactococcus lactis* on coffee aroma. This is an area which should be intensively researched upon for coffee aroma modulation.

6. Conclusion

In conclusion, the influence of numerous variables and processes during coffee fermentation on aroma formation during roasting highlights the intricate and delicate relationship between coffee fermentation and flavor. Based on the literature reviewed, improvements to the sensory qualities of coffee aroma brought about by fermentation during coffee processing is most likely attributed to the modification of the composition of aroma precursors in green coffee beans observed following fermentation. However, as fermentation in coffee processing relies on natural microflora that are present in coffee cherries, there are issues of inconsistency and uncontrollability. In view of this, there are numerous studies on the optimization of the fermentation process in coffee processing. Furthermore, numerous unconventional avenues in which fermentation was employed for coffee aroma modulation have been reported in the literature. Digestive bioprocessing was one such avenue which is responsible for the production of Kopi Luwak, one of the most expensive coffees in the world. Such exotic coffees have been associated with numerous desirable traits such as floral and fruity attributes with decreased bitterness and acidity. Among the patents that were reviewed, the employment of solid-state fermentation of green coffee beans for coffee aroma modulation is a novel area that should be explored further, especially with microorganisms commonly used for aroma development in other food products.

References

Agate, A. D., & Bhat, J. V. (1966). Role of pectinolytic yeasts in the degradation of mucilage layer of *Coffea robusta* cherries. *Applied Microbiology, 14*(2), 256–260.

Ahmad, R., & Magan, N. (2002). Microflora contamination and hydrolytic enzyme differences between monsooned and non-monsooned coffees. *Letters in Applied Microbiology, 34*(4), 279–282.

Ahmad, R., Tharappan, B., & Bongirwar, D. R. (2003). Impact of gamma irradiation on the monsooning of coffee beans. *Journal of Stored Products Research, 39*(2), 149–157.

Amrani-Hemaimi, M., Cerny, C., & Fay, L. B. (1995). Mechanisms of formation of alkylpyrazines in the Maillard reaction. *Journal of Agricultural and Food Chemistry, 43*(11), 2818–2822.

Arruda, N. P., Hovell, A. M. C., Rezende, C. M., Freitas, S. P., Couri, S., & Bizzo, H. R. (2012). Correlation between precursors and volatiles in Brazilian Arabica coffee processed by dry, semi-dry and wet methods and discrimination by principle component analysis. *Quimica Nova, 35*(10), 2044–2051.

Avallone, S., Guyot, B., Michaux-Ferriere, N., Guiraud, J. P., Olguin Palacios, E., & Brillouet, J. M. (1999). Cell wall polysaccharides of coffee bean mucilage. Histological characterization during fermentation. In *Proceedings of the 18th International Scientific Colloquium on coffee – Post-harvest treatment* (pp. 463–470). Helsinki, Finland: Association Scentifique Internationale pour le Café.

Avallone, S., Guiraud, J.-P., Guyot, B., Olguin, E., & Brillouet, J.-M. (2006). Polysaccharide constituents of coffee-bean mucilage. *Journal of Food Science, 65*(8), 1308–1311.

Avallone, S., Guyot, B., Brillouet, J. M., Olguin, E., & Guiraud, J. P. (2001). Microbiological and biochemical study of coffee fermentation. *Current Microbiology, 42*(4), 252–256.

Baensch, J., Rippstein, M., & Wood, R. D. (1996) *European Patent No. EP0791296 B1*. Munich, Germany: European Patent Office.

Baggenstoss, J., Poisson, L., Kaegi, R., Perren, R., & Escher, F. (2008). Coffee roasting and aroma formation: Application of different time–temperature conditions. *Journal of Agricultural and Food Chemistry, 56*(14), 5836–5846.

Belitz, H.-D., Grosch, W., & Schieberle, P. (2009). *Food chemistry*. Berlin, Heidelberg: Springer.

Bhumiratana, N., Adhikari, K., & Chambers, E. (2011). Evolution of sensory aroma attributes from coffee beans to brewed coffee. *LWT – Food Science and Technology, 44*(10), 2185–2192.

Bicchi, C., Iori, C., Rubiolo, P., & Sandra, P. (2002). Headspace sorptive extraction (HSSE), stir bar sorptive extraction (SBSE), and solid phase microextraction (SPME) applied to the analysis of roasted Arabica coffee and coffee brew. *Journal of Agricultural and Food Chemistry, 50*(3), 449–459.

Blank, I., Sen, A., & Grosch, W. (1992). Potent odorants of the roasted powder and brew of Arabica coffee. *Zeitschrift für Lebensmittel-Untersuchung und –Forschung, 195*(3), 239–245.

Boniello, B., Gum Jr, E., Scarpellino, R., Doonan, B., Patterson, J., & Bertkau, G. (1989) *US Patent No. 4867992*. Washington, DC: U.S. Patent and Trademark Office.

Bytof, G., Knopp, S.-E., Schieberle, P., Teutsch, I., & Selmar, D. (2005). Influence of processing on the generation of γ-aminobutyric acid in green coffee beans. *European Food Research and Technology, 220*(3–4), 245–250.

Cheong, M. W., Tong, K. H., Ong, J. J. M., Liu, S. Q., Curran, P., & Yu, B. (2013). Volatile composition and antioxidant capacity of Arabica coffee. *Food Research International, 51*(1), 388–396.

Chin, S.-T., Eyres, G. T., & Marriott, P. J. (2011). Identification of potent odourants in wine and brewed coffee using gas chromatography–olfactometry and comprehensive two-dimensional gas chromatography. *Journal of Chromatography A, 1218*(42), 7487–7498.

Ciampa, A., Renzi, G., Taglienti, A., Sequi, P., & Valentini, M. (2010). Studies on coffee roasting process by means of nuclear magnetic resonance spectroscopy. *Journal of Food Quality, 33*(2), 199–211.

Czerny, M., & Grosch, W. (2000). Potent odorants of raw Arabica coffee. Their changes during roasting. *Journal of Agricultural and Food Chemistry, 48*(3), 868–872.

Czerny, M., Mayer, F., & Grosch, W. (1999). Sensory study on the character impact odorants of roasted Arabica coffee. *Journal of Agricultural and Food Chemistry, 47*(2), 695–699.

De Maria, C. A. B., Trugo, L. C., Aquino Neto, F. R., Moreira, R. F. A., & Alviano, C. S. (1996). Composition of green coffee water-soluble fractions and identification of volatiles formed during roasting. *Food Chemistry, 55*(3), 203–207.

de Melo Pereira, G. V., Soccol, V. T., Pandey, A., Medeiros, A. B., Andrade Lara, J. M., Gollo, A. L., et al. (2014). Isolation, selection and evaluation of yeasts for use in fermentation of coffee beans by the wet process. *International Journal of Food Microbiology, 188*, 60–66.

Dorfner, R., Ferge, T., Kettrup, A., Zimmermann, R., & Yeretzian, C. (2003). Real-time monitoring of 4-vinylguaiacol, guaiacol, and phenol during coffee roasting by resonant laser ionization time-of-flight mass spectrometry. *Journal of Agricultural and Food Chemistry, 51*(19), 5768–5773.

Duarte, G. S., Pereira, A. A., & Farah, A. (2010). Chlorogenic acids and other relevant compounds in Brazilian coffees processed by semi-dry and wet post-harvesting methods. *Food Chemistry, 118*(3), 851–855.

Duboc, P., & Milo, C. (2003) *European Patent No. EP 1527695A1*. Munich, Germany: European Patent Office.

Eom, I.-Y., & Jung, M.-J. (2013). Identification of coffee fragrances using needle trap device-gas chromatograph/mass spectrometry (NTD-GC/MS). *Bulletin of the Korean Chemical Society, 34*(6), 1703–1707.

Evangelista, S. R., da Cruz Pedrozo Miguel, M. G., de Souza Cordeiro, C., Silva, C. F., Marques Pinheiro, A. C., & Schwan, R. F. (2014). Inoculation of starter cultures in a semi-dry coffee (*Coffea arabica*) fermentation process. *Food Microbiology, 44*, 87–95.

Evangelista, S. R., Silva, C. F., Miguel, M. G. P. D. C, Cordeiro, C. d. S., Pinheiro, A. C. M., Duarte, W. F., et al. (2014). Improvement of coffee beverage quality by using selected yeasts strains during the fermentation in dry process. *Food Research International, 61*, 183–195.

Farah, A., Monteiro, M. C., Calado, V., Franca, A. S., & Trugo, L. C. (2006). Correlation between cup quality and chemical attributes of Brazilian coffee. *Food Chemistry, 98*(2), 373–380.

Food and Agriculture Organization. (2006). Fermentation of coffee – Control of operation. In *Good hygiene practices along the coffee chain*.

Franca, A. S., Mendonça, J. C. F., & Oliveira, S. D. (2005). Composition of green and roasted coffees of different cup qualities. *LWT – Food Science and Technology, 38*(7), 709–715.

Franca, A. S., Oliveira, L. S., Mendonça, J. C. F., & Silva, X. A. (2005). Physical and chemical attributes of defective crude and roasted coffee beans. *Food Chemistry, 90*(1–2), 89–94.

Gloess, A. N., Schönbächler, B., Klopprogge, B., D'Ambrosio, L., Chatelain, K., Bongartz, A., et al. (2013). Comparison of nine common coffee extraction methods: Instrumental and sensory analysis. *European Food Research and Technology, 236*(4), 607–627.

Gonzalez-Rios, O., Suarez-Quiroz, M. L., Boulanger, R., Barel, M., Guyot, B., Guiraud, J.-P., et al. (2007a). Impact of "ecological" post-harvest processing on coffee aroma: II. Roasted coffee. *Journal of Food Composition and Analysis, 20*(3–4), 297–307.

Gonzalez-Rios, O., Suarez-Quiroz, M. L., Boulanger, R., Barel, M., Guyot, B., Guiraud, J.-P., et al. (2007b). Impact of "ecological" post-harvest processing on the volatile fraction of coffee beans: I. Green coffee. *Journal of Food Composition and Analysis, 20*(3–4), 289–296.

International Coffee Organization (2014a). *World coffee trade (1963–2013): A review of the markets, challenges and opportunities facing the sector*. London: United Kingdom.

International Coffee Organization. Breakdown of exports of green Arabica and green Robusta for countries exporting significant volumes of both types of coffee. Retrieved from <http://dev.ico.org/prices/m1a.htm>, 2014b (accessed 24.03.2015).

Jackels, S. C., & Jackels, C. F. (2005a) *U.S. Patent No. US20060204620*. Washington, DC: U.S. Patent and Trademark Office.

Jackels, S. C., Jackels, C. F., Vallejos, C., Kleven, S., Rivas, R., & Fraser-Dauphinee, S. (2006). Control of the coffee fermentation process and quality of resulting roasted coffee: Studies in the field laboratory and on small farms in Nicaragua during the 2005–06 harvest. In *21st International Scientific Colloquium on Coffee – Post-harvest processing and green coffee quality*. Montpellier, France.

Jackels, S. C., & Jackels, C. F. (2005b). Characterization of the coffee mucilage fermentation process using chemical indicators: A field study in Nicaragua. *Journal of Food Science, 70*(5), C321–C325.

Joët, T., Laffargue, A., Descroix, F., Doulbeau, S., Bertrand, B., de Kochko, A., et al. (2010). Influence of environmental factors, wet processing and their interactions on the biochemical composition of green Arabica coffee beans. *Food Chemistry, 118*(3), 693–701.

Knopp, S., Bytof, G., & Selmar, D. (2005). Influence of processing on the content of sugars in green Arabica coffee beans. *European Food Research and Technology, 223*(2), 195–201.

Lee, C., Lee, Y., Lee, J.-G., & Buglass, A. J. (2013). Development of a simultaneous multiple solid-phase microextraction-single shot-gas chromatography/mass spectrometry method and application to aroma profile analysis of commercial coffee. *Journal of Chromatography A, 1295*, 24–41.

Leloup, V., Gancel, C., Liardon, R., Rytz, A., & Pithon, A. (2004). Impact of wet and dry process on green coffee composition and sensory characteristics. In *ASIC proceedings of 20th Colloque coffee* (pp. 93–100). Association Scentifique Internationale pour le Cafe: Bangalore, India.

Li, P.-J., Li, C.-C., & Li, C.-H. (2010) *U.S. Patent No. US 20100239711A1*. Washington, DC: U.S. Patent and Trademark Office.

Lin, C. C. (2010). Approach of improving coffee industry in Taiwan – Promote quality of coffee bean by fermentation. *The Journal of International Management Studies, 5*(1), 154–159.

Lopez, C. I., Bautista, E., Moreno, E., & Dentan, E. (1989). Factors related to the formation of "overfermented coffee beans" during the wet processing method and storage of coffee. In *Proceedings of the 13th International Scientific Colloquium on Coffee* (pp. 373–384). Association for Science and Information on Coffee: Paipa, Columbia.

Lopez-Galilea, I., Fournier, N., CID, C., & Guichard, E. (2006). Changes in headspace volatile concentrations of coffee brews caused by the roasting process and the brewing procedure. *Journal of Agricultural and Food Chemistry, 54*(22), 8560–8566.

Low, M. Y., Parker, J. K., & Mottram, D. S. (2007). Mechanisms of alkylpyrazine formation in a potato model system containing added glycine. *Journal of Agricultural and Food Chemistry, 55*(10), 4087–4094.

Maeztu, L., Sanz, C., Andueza, S., Paz De Pena, M., Bello, J., & Cid, C. (2001). Characterization of expresso coffee aroma by static headspace GC–MS and sensory flavor profile. *Journal of Agricultural and Food Chemistry, 49*(11), 5437–5444.

Main, D. The world's most expensive coffee comes from elephant poo. *Newsweek*. Retrieved from <http://www.newsweek.com/worlds-most-expensive-coffee-comes-elephant-poo-266301>, 2014, August 22 (accessed on 12.03.2015).

Marcone, M. F. (2004). Composition and properties of Indonesian palm civet coffee (Kopi Luwak) and Ethiopian civet coffee. *Food Research International, 37*(9), 901–912.

Marshall, E., & Mejia, D. (2011). *Traditional fermented food and beverages for improved likelihood*. Rome: Food and Agriculture Organization of the United Nations.

Masoud, W., Cesar, L. B., Jespersen, L., & Jakobsen, M. (2004). Yeast involved in fermentation of *Coffea arabica* in East Africa determined by genotyping and by direct denaturating gradient gel electrophoresis. *Yeast, 21*(7), 549–556.

Masoud, W., & Kaltoft, C. H. (2006). The effects of yeasts involved in the fermentation of Coffea arabica in East Africa on growth and ochratoxin A (OTA) production by *Aspergillus ochraceus*. *International Journal of Food Microbiology, 106*(2), 229–234.

Mayer, F., Czerny, M., & Grosch, W. (2000). Sensory study of the character impact aroma compounds of a coffee beverage. *European Food Research and Technology, 211*(4), 272–276.

Mayer, F., & Grosch, W. (2001). Aroma simulation on the basis of the odourant composition of roasted coffee headspace. *Flavour and Fragrance Journal, 16*(3), 180–190.

Montavon, P., Mauron, A.-F., & Duruz, E. (2003). Changes in green coffee protein profile during roasting. *Journal of Agricultural and Food Chemistry, 51*(8), 2335–2343.

Mussatto, S. I., Machado, E. M. S., Martins, S., & Teixeira, J. A. (2011). Production, composition, and application of coffee and its industrial residues. *Food and Bioprocess Technology, 4*(5), 661–672.

Pederson, C. S., & Breed, R. S. (1946). Fermentation of coffee. *Journal of Food Science, 11*(2), 99–106.

Petisca, C., Pérez-Palacios, T., Farah, A., Pinho, O., & Ferreira, I. M. P. L. V. O. (2013). Furans and other volatile compounds in ground roasted and espresso coffee using headspace solid-phase microextraction: Effect of roasting speed. *Food and Bioproducts Processing, 91*(3), 233–241.

Poisson, L., Schmalzried, F., Davidek, T., Blank, I., & Kerler, J. (2009). Study on the role of precursors in coffee flavor formation using in-bean experiments. *Journal of Agricultural and Food Chemistry, 57*(21), 9923–9931.

Puerta-Quintero, G. I. (2001). Strategies to gurantee the quality of the beverage in Colombian coffees. In *Proceedings of the 19th International Scientific Colloquium on Coffee*. Trieste, Italy: Association for Science and Information on Coffee.

Rothfos, N. B. (1985). *Coffee production*. Hamburg: Gordian-Max-Rieck.

Schwan, R. F., & Wheals, A. E. (2003). Mixed microbial fermentations of chocolate and coffee. In T. Boekhout & V. Robert (Eds.), *Yeasts in food* (pp. 429–449). Germany: Behr's Verlag.

Selmar, D., Bytof, G., Knopp, S. E., & Breitenstein, B. (2006). Germination of coffee seeds and its significance for coffee quality. *Plant Biol (Stuttg), 8*(2), 260–264.

Selmar, D., Bytof, G., & Knopp, S. (2002). New aspects of coffee processing: The relation between seed germination and coffee quality. In *Proceedings of the international congress of ASIC* (pp. 14–18). Paris, Trieste.

Shigeru, H., Toru, K., & Keiji, I. (1992) *Japan Patent No. JP H04278072 A*. Japan.

Silva, C. F., Batista, L. R., & Schwan, R. F. (2008). Incidence and distribution of filamentous fungi during fermentation, drying and storage of coffee (*Coffea Arabica* L.) beans. *Brazilian Journal of Microbiology, 39*(3), 521–526.

Silva, C. F., Schwan, R. F., Sousa Dias, E., & Wheals, A. E. (2000). Microbial diversity during maturation and natural processing of coffee cherries of *Coffea arabica* in Brazil. *International Journal of Food Microbiology, 60*(2–3), 251–260.

Silva, C. F., Vilela, D. M., de Souza Cordeiro, C., Duarte, W. F., Dias, D. R., & Schwan, R. F. (2013). Evaluation of a potential starter culture for enhance quality of coffee fermentation. *World Journal of Microbiology and Biotechnology, 29*(2), 235–247.

Small, L. E., & Asquith, T. N. (1989) *European Patent No. EP0337541 A3*. Munich, Germany: European Patent Office.

Sunarharum, W. B., Williams, D. J., & Smyth, H. E. (2014). Complexity of coffee flavor: A compositional and sensory perspective. *Food Research International, 62*, 315–325.

Tarzia, A., dos Santos Scholz, M. B., & de Oliveira Petkowicz, C. L. (2010). Influence of the postharvest processing method on polysaccharides and coffee beverages. *International Journal of Food Science & Technology, 45*(10), 2167–2175.

Van Pee, W., & Castelein, J. M. (1972). Study of the pectinolytic microflora, particularly the *Enterobacteriaceae*, from fermenting coffee in the congo. *Journal of Food Science, 37*, 171–174.

Velmourougane, K. (2011). Effects of wet processing methods and subsequent soaking of coffee under different organic acids on cup quality. *World Journal of Science and Technology, 1*(7), 32–38.

Velmourougane, K. (2012). Impact of natural fermentation on physicochemical, microbiological and cup quality characteristics of Arabica and Robusta Coffee. *Proceedings of the National Academy of Sciences, India Section B: Biological Sciences, 83*(2), 233–239.

Wong, K. H., Abdul Aziz, S., & Mohamed, S. (2008). Sensory aroma from Maillard reaction of individual and combinations of amino acids with glucose in acidic conditions. *International Journal of Food Science & Technology, 43*(9), 1512–1519.

Yeretzian, C., Jordan, A., Badoud, R., & Lindinger, W. (2002). From the green bean to the cup of coffee: Investigating coffee roasting by on-line monitoring of volatiles. *European Food Research and Technology, 214*(2), 92–104.